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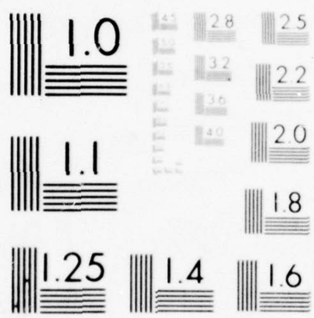
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ACUTE TOXICITY OF DIISOPROPYLMETHYL PHOSPHONATE AND DICYCLOPENTADIENE TO AQUATIC ORGANISMS

BY

R.E. BENTLEY, G.A. LEBLANC, T.A. HOLLISTER, AND B.H. SLEIGHT, III

FINAL REPORT

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute toxicity of diisopropylmethyl phosphonate (DIMP) and dicyclopentadiene (DCPD) was studied utilizing aquatic organisms representing several trophic levels in an aquatic ecosystem. DCPD was found to be approximately 10X more toxic than DIMP. The eggs and 7-day old fry of the fathead minnow were the life stages least susceptible to DCPD and DIMP, respectively. Increasing hardness and pH 8.0 appeared to decrease slightly the toxicity of DIMP while the toxicity of DCPD was not significantly altered by varying

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20. Abstract (continued)

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water quality parameters. Generally, aging of solutions had little affect on the toxicity of DCPD. A 50% decrease in toxicity to bluegill was observed for DIMP solutions allowed to age for 96 hours. Essentially no bioconcentration (<1X) was observed for bluegill continually exposed to ^{14}C-DIMP, and the estimated maximum bioconcentration factor for ^{14}C-DCPD was 53X. Based on an application factor of 0.05, and an LC50 of 257 mg/ℓ observed for the most sensitive aquatic organism tested (bluegill at 25^{o}C), a water quality criterion of 12.5 mg/ℓ DIMP is recommended for the protection of freshwater aquatic life. Based on an application factor of 0.05, and an LC50 of 10.5 observed for the most sensitive aquatic organism tested (Daphnia magna), a water quality criterion of 0.5 mg/ℓ DCPD is recommended for the protection of freshwater aquatic life.

SUMMARY

Of the two compounds studied, DCPD was the more (ca 10X) toxic to aquatic organisms during static bioassays with 96-hour LC50's ranging from 15.7 mg/ℓ to 31.1 mg/ℓ. The egg of the fathead minnow was the life stage least susceptible to DCPD exposure, while in the DIMP life stage bioassays, the 7-day old fry exhibited the highest degree of tolerance. Variations in water quality parameters appeared to decrease the toxicity of DIMP at elevated hardnesses, and at pH 8.0. In general, the water quality conditions did not significantly alter the toxicity of DCPD to bluegill. A 50% decrease in toxicity to bluegill was observed for the DIMP solution allowed to stand for 96 hours. Aging for 96 hours appeared to have little effect on the toxicity of DCPD to bluegill. Finally, essentially no bioconcentration (<1X) was observed for those bluegill exposed to ^{14}C-DIMP, while the estimated maximum bioconcentration factor for ^{14}C-DCPD is 53X. Based on an application factor of 0.05, and an LC50 of 257 mg/ℓ observed for the most sensitive aquatic organism tested (bluegill at 25°C), a water quality criterion of 12.5 mg/ℓ DIMP is recommended for the protection of freshwater aquatic life. Based on an application factor of 0.05 and an LC50 of 10.5 mg/ℓ for the most sensitive aquatic organism tested (*Daphnia magna*), a water quality criterion of 0.5 mg/ℓ DCPD is recommended for the protection of freshwater aquatic life.

CRITERIA FORMULATION

DIMP

The acute toxicity (LC50 and EC50) of diisopropylmethyl phosphonate to a wide variety of aquatic organisms representing several trophic forms (including primary producer organisms, primary consumers, and secondary consumers) under a wide variety of water quality conditions ranged from 257-6,332 mg/ℓ.

In lieu of a specific laboratory-derived application factor based on chronic toxicity studies, an appropriate application factor (e.g., 0.1, 0.05, 0.01) must be utilized to estimate safe concentrations during chronic exposure. The selection of an appropriate application factor must consider persistence, cumulative toxic effects, and bioaccumulation potential of the chemical. Acute toxicity studies with bluegill and aged solutions of DIMP indicate that after only 96 hours, acute toxicity of DIMP in water is less than 1/3-1/2 of that observed with fresh solutions suggesting a lack of persistence of DIMP in water. During continuous exposure of bluegill to 150 mg/ℓ DIMP for 14 days, no toxic effects were evident, indicating a lack of cumulative toxicity associated with this chemical. Finally, a pilot bioconcentration study with ^{14}C-DIMP demonstrated no bioaccumulation of the chemical by bluegill.

Based on these observations, it would appear that, in lieu of adequate chronic toxicity data, 0.05 probably represents a reasonable application factor for estimating safe concentrations of DIMP in water.

Based on an application factor of 0.05, and an LC50 of 257 mg/ℓ observed for the most sensitive aquatic organisms tested (bluegill, at 25°C), a water quality criterion of 12.5 mg/ℓ DIMP is recommended for the protection of freshwater aquatic life.

DCPD

The acute toxicity (LC50 and EC50) of dicyclopentadiene to a wide variety of aquatic organisms representing several trophic forms (including primary producer organisms, primary consumers and secondary consumers), under a wide variety of water quality conditions ranged from 10.5-120 mg/ℓ.

In lieu of a specific laboratory-derived application factor based on chronic toxicity studies, an appropriate application factor must be utilized to estimate safe concentrations during chronic exposure. Acute toxicity studies with bluegill indicate that the toxicity of DCPD solutions after 96 hours of aging is somewhat less than that of the fresh solutions. During continuous exposure of bluegill for 14 days to approximately

1 mg/ℓ ^{14}C-DCPD, no toxic effects were observed. During this pilot bioconcentration study, maximum bioaccumulation (ca 50X) occurred during the initial 96 hours of exposure. More significantly, ^{14}C-residue concentrations in fish tissue declined dramatically despite continued exposure through 14 days, a phenomenon possibly mediated through an enzyme induction process. Based on these observations, and in lieu of adequate chronic toxicity data, 0.05 probably represents a reasonable application factor for estimated safe concentrations of DCPD in water.

Based on an application factor of 0.05, and an LC50 of 10.5 mg/ℓ for the most sensitive aquatic organism tested (Daphnia), a water quality criterion of 0.5 mg/ℓ is recommended for the protection of freshwater aquatic life.

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INTRODUCTION

The primary objectives of these studies were to provide the U.S. Army Medical Research and Development Command with the necessary information for DIMP and DCPD to evalute the relative susceptibility of phytoplankton, aquatic invertebrates, and fishes to acute exposure (Task 1), to evaluate the relative sensitivity of specific life history stages of fathead minnow to acute exposure (Task 2), to evaluate the effects of water quality on acute toxicity (Task 3), to provide information on the short-term stability of the toxicological characteristics of the test compounds in water (Task 4), and to provide information on the bioconcentration by fish of DIMP and DCPD from water (Task 5).

The phytoplankton studies were conducted at the Marine Research Laboratory of E G & G, Bionomics, Pensacola, Florida. The aquatic invertebrate and fish studies were conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

MATERIALS AND METHODS

Test Materials

The materials evaluated during these bioassays were diisopropylmethyl phosphonate (DIMP) and dicyclopentadiene (DCPD), both clear liquids, received from Richmond Organics, Richmond, Virginia, and Aldrich Chemical Company, Milwaukee, Wisconsin, respectively (Table 1). The DIMP was tested on a 98.5% active ingredient basis, and the DCPD was tested on a 95% active ingredient basis. Nominal test concentrations are reported as milligrams active ingredient per liter. The DIMP appeared miscible with both the culture medium used in the phytoplankton assays and the water used in preparing stock solutions, and therefore, was added to test water directly without a solvent/carrier. The DCPD was not miscible with either the culture medium or water. Therefore, stock solutions were prepared in a solution of reagent-grade acetone. The stock solution used in the phytoplankton assays was prepared in the ratio of 1 part DCPD:99 parts acetone (volume:volume), and that used in the fish bioassays was prepared in the ratio of 1.5 parts DCPD:98.5 parts acetone (volume:volume). The stock solution used in the macroinvertebrate bioassays was prepared by adding 1 part DCPD and 2 parts acetone to 997 parts of water, and mixed with a magnetic stirrer.

Test Organisms

Algae tested were the cyanophytes (blue-greens) Microcystis aeruginosa and Anabeana flos-aquae; the chlorophyte (green) Selenastrum capricornutum; and the chrysophyte (diatom) Navicula pelliculosa. The cultures were obtained from the algae collection at the University of Indiana, Bloomington, Indiana and the Pacific Northwest Water Quality Laboratory (EPA), Corvallis, Oregon. Each alga was maintained in stock cultures at Bionomics Marine Research Laboratory. These cultures were maintained according to the methods outlined in the Algal Assay Procedure: Bottle Test (U.S. EPA, 1971).

The invertebrate species tested were water flea (Daphnia magna), midge (Chironomus tentans), scud (Gammarus fasciatus), and sowbug (Asellus militaris). The water flea ($\leq$ 24 hours old) and midge (2nd-3rd instar) were from Bionomics Aquatic Toxicology Laboratory populations cultured in static, aerated well water, with a hardness of 35 milligrams per liter (mg/ℓ) as $CaCO_3$, a pH of 7.1, a temperature of 21 $\pm$ 1.0°C and a dissolved oxygen (DO) concentration of greater than 60% of saturation. The scud and sowbug were collected locally in Massachusetts by laboratory personnel and held at Bionomics Aquatic Toxicology Laboratory in flowing well water for at least two weeks prior to testing.

Bluegill (Lepomis macrochirus) used in these bioassays were obtained from a commercial fish hatchery in Nebraska and had a mean and standard deviation (N=30) wet weight of 1.1 $\pm$ 0.35 grams (g) and standard length of 31 $\pm$ 8.6 millimeters (mm). The channel catfish (Ictalurus punctatus), acquired from a commercial fish farmer in Missouri, had a mean and standard deviation (N=30) wet weight of 1.3 $\pm$ 0.56 g and standard length of 36 $\pm$ 7.6 mm. The fathead minnow (Pimephales promelas) were obtained from a commercial fish farmer in Arkansas and had a mean and standard deviation (N=30) wet weight of 1.4 $\pm$ 0.55 g and standard length of 36 $\pm$ 8.6 mm. The rainbow trout (Salmo gairdneri) were acquired from a commercial fish hatchery in Washington and had a mean and standard deviation (N=30) wet weight of 1.6 $\pm$ 0.46 g and standard length of 38 $\pm$ 8.6 mm. All test fish were held in 1700-ℓ concrete raceways which were coated with an epoxy resin paint to prevent leaching of extraneous materials into the water. Well water, of the same characteristics described previously, at a temperature of 21 $\pm$ 1.0°C for bluegill, channel catfish, and fathead minnow, and 14 $\pm$ 1.0°C for rainbow trout, flowed through these raceways at a minimum of 4 liters/minute which provided an adequate rate of turnover for maintaining these species. Test animals were maintained under these conditions for a minimum of 30 days prior to use in the bioassays. During the holding period, fish mortality was less than 2%. Test animals used in the

bioassays were from the same year class. The standard length of the longest fish was no more than twice that of the shortest. Immediately prior to use in these tests, fish were acclimated to test conditions of temperature and water quality over a 48-hour period. Fish were not fed during this acclimation period, there was no mortality observed during this period and fish appeared to be in excellent condition at the initiation of the tests.

Test Conditions

Phytoplankton assay procedures followed the Algal Assay Procedure: Bottle Test (U.S. EPA, 1971). Effects of the compounds on test algae were measured by determining *in vivo* chlorophyll *a* content at 24, 48 and 96 hours of exposure and cell numbers at 96 hours as compared to controls. The *in vivo* chlorophyll *a* of cultures was measured with a Turner Model III fluorometer. All cell counts were made using a hemacytometer and Zeiss Standard 14 compound microscope.

The methodology for conducting the macroinvertebrate and fish toxicity tests followed the recommended bioassay procedures as described in the "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975). These methods reflect the most recent state-of-the-art metho-

dology for acute toxicity studies with aquatic organisms. The macroinvertebrate bioassays were conducted in 250 milliliter (mℓ) beakers which contained 166 mℓ of test solution. Diluent water used in these tests was aged for at least 24 hours prior to initiation of the tests. For each concentration, the appropriate amount of the test compound was pipetted into 500 mℓ of diluent water and mixed with a magnetic stirrer. This solution was then divided into three equal aliquots in triplicate beakers to provide replicate exposure treatments. All beakers were maintained at 20 $\pm$ 1.0^{o}C and test solutions were not aerated during the test. Five animals were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 15 test organisms per concentration per test.

Static fish bioassays were conducted in 19.6 liter glass vessels held in constant temperature water baths at 21 $\pm$ 1.0^{o}C for the bluegill, channel catfish, and fathead minnow and at 14 $\pm$ 1.0^{o}C for the rainbow trout. The standard diluent (well water) used in this test had the same water quality characteristics as described previously for holding water.

Statistical Analyses

Results for all tests were expressed as the median effective

concentration (EC50) for phytoplankton, and the median lethal concentration (LC50) for the macroinvertebrates and fishes. The criteria utilized in bioassays was chlorophyll a content and cell number for phytoplankton, and death for invertebrates and fishes. The EC50 and LC50 values and their 95% confidence intervals were calculated by transforming the test concentrations and the corresponding observed percentage response to logarithms and probits, respectively. The transformed data were then utilized in a least squares regression analysis and EC50 and LC50 values (confidence limits) were estimated from the calculated regression equation.

Task 1 - Relative Susceptibility of Aquatic Species

As stated previously, the DIMP appeared to be miscible with the algae culture medium at all concentrations tested; the DCPD was immiscible at all concentrations, and was therefore prepared with reagent grade acetone. Using this acetone stock solution, at concentrations greater than 100 mg/ℓ, the DCPD was observed to come out of solution as evidenced by the presence of droplets of what appeared to be DCPD throughout the culture medium. As a result, 100 mg/ℓ was the highest concentration of DCPD tested. Since acetone was used as a solvent/carrier, preliminary tests were conducted to determine its affect on chlorophyl a concentrations and cell numbers

of test algae. Concentrations of acetone tested were 100 and 1,000 mg/ℓ. These were the minimum and maximum amounts added to the test cultures to obtain desired concentrations of DCPD.

In both the macroinvertebrate and fish bioassays, negative controls, which consisted of the same dilution water and conditions as test concentrations, but with no DIMP or DCPD, were conducted. Additionally, positive controls, which contained a volume of acetone equivalent to the greatest amount of the solvent introduced into any test vessel, were also maintained for the DCPD bioassays.

During the fish bioassays, the dissolved oxygen (DO) concentration, pH and temperature of test solutions were checked at 0, 48 and 96 hours of exposure in two selected test concentrations, at a minimum. DO and temperature were measured with a YSI dissolved oxygen meter and combination oxygen-temperature probe; pH was measured with a Corning Digital pH meter and probe.

Task 2 - Susceptibility of Life Stages of Fish

Fathead minnow were chosen as the test species for Task 2 because of the ability to readily rear their various life stages

in the laboratory. The susceptibilities of selected life stages (egg, 1-hour old, newly-hatched fry, 7-day old fry, 30-day old fry, and 60-day old fry) of fathead minnow to DIMP and DCPD were evaluated under static bioassay conditions over a 144-hour period for the eggs, and over a 96-hour period with all other life stages. The egg bioassays were conducted in 250 mℓ beakers containing 200 mℓ of solution (10 eggs/beaker, 3 replicates/concentration). The 1-hour old fry and 7-day old fry bioassays were also conducted in 250 mℓ beakers containing 200 mℓ of solution (10 animals/beaker, duplicate beakers). The 30-day old fry and 60-day old fry bioassays were conducted in 1-gallon glass jars containing 3 ℓ of solution (10 fry/jar, duplicate jars). The LC50 values for the egg tests were calculated at 24, 48 and 144 hours of exposure. The time period of 144 hours allowed 100% hatch of eggs in all control beakers. In addition to percentage mortalities, percentage hatch was also observed. All of the life stage tests were conducted at 25 $\pm$ 1.0°C. Dissolved oxygen values were measured in randomly selected test vessels during these bioassays.

Task 3 - Impact of Environmental Factors on Toxicity

Due to the sensitivity of bluegill to DIMP and DCPD determined during Tasks 1 and 2, the availability of these fish and the expected presence of this species in most of those areas where

the chemical compounds might be found, bluegill were selected as the test species for this task. The susceptibility of bluegill to the chemical compounds under various water quality conditions was evaluated under static bioassay conditions for a 96-hour period. The bluegill used in these tests were obtained from a commercial fish farmer in Nebraska and had a mean and standard deviation (N=30) wet weight and standard length of 1.1 $\pm$ 0.35 g and 30 $\pm$ 8.6 mm, respectively. Bioassays were conducted utilizing bluegill to determine the 24-, 48- and 96-hour LC50 values of DIMP and DCPD: a) at three temperatures representing the lower end (15^{o}C), mid-point (20^{o}C), and upper end (25^{o}C) of the normal temperature range for bluegill using soft water (35 mg/ℓ $CaCO_3$) at neutral pH; b) in soft water (35 mg/ℓ $CaCO_3$), in hard water (100 mg/ℓ $CaCO_3$) and in very hard water (250 mg/ℓ $CaCO_3$) using water of pH 7.0 at the recommended test temperature of 20^{o}C; and c) at a pH of 6.0, 7.0 and 8.0 using standard soft water at the recommended test temperature of 20^{o}C. The diluent for each of these conditions was prepared as recommended by Marking and Dawson (1973) (Tables 2a and 2b). Dissolved oxygen values were measured in various test vessels during these bioassays.

Task 4 - Stability of Toxicological Properties

The susceptibility of bluegill to DIMP and DCPD was evaluated under static bioassay conditions for a 96-hour period utilizing

"aged" (0, 12, 24, 48 and 96 hours) solutions of each compound. The bluegill used in these tests were acquired from a commercial fish farmer in Nebraska, and had a mean and standard deviation (N=30) wet weight of 1.1 $\pm$ 0.35 g and standard length of 31 $\pm$ 8.6 mm. Dissolved oxygen values were monitored at various intervals throughout the test in selected concentrations to determine if there was an oxygen demand by the compound.

Task 5 - Bioconcentration

Bluegill were chosen as the test species due to their availability and their general acceptance as the species of choice in bioconcentration tests. Bluegill, having a mean and standard deviation (N=30) wet weight of 1.75 $\pm$ 0.65 g and standard length of 36.1 $\pm$ 5.5 mm, were obtained from a commercial fish farmer in Connecticut. These fish were held under those conditions previously described for at least 30 days prior to the initiation of the studies. The studies were conducted using a modification of a proportional dilution apparatus (Mount and Brungs, 1967) which provided for the automatic, intermittent introduction of the test material and diluent water into the test chamber. Three 30-liter experimental units were utilized in the system.

The DIMP and DCPD used in the ^{14}C-accumulation study were

received as clear liquids contained in sealed screw-cap vials from Litton Bionetics, Inc. Correspondence which accompanied these vials identified their contents as: methyl-carbon labeled ^{14}C-DIMP, 100 μCi (25 microliters; μℓ) and uniformly ring-labeled ^{14}C-DCPD, 100 μCi (50 μℓ).

The contents of the vial containing ^{14}C-DIMP and an additional 479 mg of unlabeled DIMP were quantitatively transferred to a 1-liter volumetric flask, and diluted to volume with distilled water. The contents of the vial containing ^{14}C-DCPD and an additional 236 mg of unlabeled DCPD were quantitatively transferred to a 1-liter volumetric flask, and diluted to volume with distilled water. To determine the specific activity of ^{14}C-DIMP, three 1 mℓ aliquots of the superstock solution were transferred to glass vials containing 15 mℓ of counting solution. These vials were placed in the liquid scintillation spectrometer and the mean specific activity was measured to be 0.50 $\pm$ 0.015 disintegrations per minute per microgram (dpm/μg), equivalent to 106% of the theoretical concentration. A similar procedure was used to determine the specific activity of ^{14}C-DCPD. The mean specific activity was measured to be 6.46 $\pm$ 0.55 dpm/μg, equivalent to 69% of the theoretical concentration.

Fifty bluegill were placed into each of three experimental units. Aerated well water having a pH of 7.1, a total hard-

ness of 35 mg/ℓ as $CaCO_3$, a dissolved oxygen concentration of greater than (>) 60% of saturation and a temperature of 18 ± 1.0°C was provided to each unit at a flow rate of 5 l/hour. Bluegill in one unit were exposed to 150 mg/ℓ of ^{14}C-DIMP, those bluegill in a second unit were exposed to 1.00 mg/ℓ ^{14}C-DCPD, and the third unit served as a control. Stock solutions for the ^{14}C-DIMP and ^{14}C-DCPD units were prepared from the superstock solutions and were mixed in distilled water and acetone, respectively. The mechanical dilution apparatus was used to establish and maintain the desired chemical concentration. Fish in all units were fed a dry pelleted ration *ad libitum* each day. During the experiment, dissolved oxygen concentrations were measured bi-weekly using the standard Winkler method (APHA *et al*., 1971, p. 474-484) and determined to be >60% of saturation. Fish remaining in the test units after 14 days were transferred to clean flowing ^{14}C-DIMP or ^{14}C-DCPD-free water for 7 days (depuration phase).

Water and bluegill were sampled from the units after 1, 2, 4, 7, 10 and 14 days of exposure. During the depuration period, fish were sampled 1, 3 and 7 days after transfer. Water was not sampled during this period.

Duplicate 5-mℓ water samples were taken directly from both units on all sample days during the exposure period. Each

sample was pipetted from the test unit into a glass vial containing 15 mℓ of counting solution.

At each sampling interval during both exposure and depuration, 3 fish were removed from each experimental unit, eviscerated, and the distribution of ^{14}C-residues in the edible (muscle) portion was investigated by radiometric analysis.

Each portion (0.75-1.50 g) of the muscle tissue from each fish sampled was air dried for approximately 24 hours in a combustion cone at 21°C. Each dried sample (0.4-1.0 g) was combusted in a Packard Model 306 Tri-Carb Sample Oxidizer. The resulting $^{14}CO_2$ was trapped as a carbonate in a mixture of Carbosorb (1M hyamine hydroxide in methanol) and scintillator cocktail (4 g, 98% PPO + 2% bis-MSB/liter toluene) in a counting vial. Standard reference material consisting of ^{14}C-methyl methacrylate (New England Nuclear Corp.) was oxidized with control fish tissue to determine recovery values of the Packard Oxidizer. Recovery values ranged from 99.6-101.3% and data were not corrected for recovery. All results were expressed as the mean plus standard error of ^{14}C-residues/g wet weight of tissue. Prior to the analyses of a set of tissue samples, the oxidizer unit was "cleaned" by consecutively burning two pressed paper discs to eliminate any residual ^{14}C-activity (memory) which could be a source of error

in analyses of tissue samples containing low levels of radioactivity.

Concentrations of ^{14}C-DIMP and ^{14}C-DCPD in the water from the treated unit were determined by directly counting the triplicate 5-mℓ samples, taken at each sample interval. The liquid scintillation fluid was composed of xylene base counting solution consisting of a nonionic surfactants with PPO + bis/ MSB surfactants.

All measurements of radioactivity were made to 7.8% maximum probable error (95% confidence interval) using a Model 2112 Packard Tri-Carb Liquid Scintillation Spectrometer. Counting efficiences (E) were determined as follows: Aliquots of a ^{14}C-toluene standard, obtained from New England Nuclear, with an accurately known specific activity were added to a series of counting vials containing scintillation cocktail of a type and volume identical to those used for counting water or combusted tissue samples. Then, increasing volumes of nitromethane, a chemical quenching agent, were added to individual vials in each series. The vials were counted with two channels simultaneously and the channel ratios determined. Counting efficiency of each standard was determined as the quotient of counts per minute divided by the disintegrations per minute of the standard. From these data, a graph of counting effi-

ciency versus channel ratio was constructed for each of the two types of samples. Counting efficiencies of all subsequent samples were determined by calculating the channel ratio and interpolating the corresponding counting efficiency from the graph (Bransome, 1970). By this method, counting efficiencies for ^{14}C-DIMP ranged from 74-77% for water and 58-63% for fish tissue. Those for ^{14}C-DCPD ranged from 68-74% for water, and 56-77% for fish tissue.

The calculations used in the analysis of the contents of a liquid scintillation counting vial were the following:

i. net counts/minute = (sample - control) cpm

ii. dpm in combusted sample $= \dfrac{\text{net cpm}}{\text{counting efficiency}}$

iii. total ^{14}C-residue calculated as DIMP or DCPD (μg/ℓ) $= \dfrac{\text{dpm of combusted sample}}{\text{specific activity of combusted DIMP or DCPD (dmp/}\mu\text{g)} \times \text{sample weight (g)}}$

Sample Calculation

$$\frac{\text{net cpm (441)/E (0.77)}}{\text{specific activity x sample wt.}\ (6.46\ \text{dpm}/\mu\text{g})\times(2.69\ \text{g})} = 32.96\ \mu\text{g/g}\ ^{14}\text{C-residues calculated as DCPD in combusted tissue}$$

Mean background levels for untreated test samples have been determined to be 41 cpm for bluegill and 40 cpm for water. Samples were counted for a maximum of 100 minutes or for a

sufficient period of time to generate a maximum total of 5,000 counts. Using this procedure, the probable error of accepting 22 cpm above mean background level as minimum detectable radiation is 7.8%. Minimum detectable limits of ^{14}C-DIMP were 100 mg/kg for fish and 7.5 mg/ℓ for water, and those for ^{14}C-DCPD were 5.00 mg/kg for fish and 0.60 mg/ℓ for water.

RESULTS AND DISCUSSION

Diisopropylmethyl phosphonate (DIMP)

A summary of those calculated EC50 and LC50 values and 95% confidence limits at termination of exposure is presented (Table 3). Based on *in vivo* chlorophyll *a* reduction, the 96-hour EC50's generally indicate a high degree of similarity in the sensitivities of *Microcystis aeruginosa*, *Selenastrum capricornutum*, and *Navicula pelliculosa* to DIMP (lot #1). EC50 values were 1,827, 2,301, and 2,045 mg/ℓ, respectively after 96 hours of exposure to DIMP. The alga *Anabeana flos-aquae* was the one least affected by DIMP (lot #1) exhibiting a 96-hour EC50 value of 6,334 mg/ℓ (Table 4).

The 96-hour EC50 values for reduction in cell numbers were similar to those for chlorophyll a reduction. These ranged from 2,345 mg/ℓ for *N. pelliculosa* to 6,107 mg/ℓ for *A. flos-aquae* (Table 5).

The toxicity of DIMP (lot #1) appeared to increase over the 96-hour exposure period for each of the algae tested (Tables 6-13). A slight stimulation of chlorophyll *a* was observed in *A. flos-aquae* after 48-hour exposures in 3,787 and 4,734 mg/ℓ.

Of the macroinvertebrate species tested, *Daphnia magna* and

Gammarus fasciatus exhibited the greatest sensitivities to DIMP (lot #1), followed by _Chironomus tentans_ and _Asellus militaris_ (Tables 14 and 15). The calculated 48-hour LC50 values were 267, 494, 1,720 and 2,160 mg/ℓ, respectively.

The concentration of dissolved oxygen measured in bioassays with all fish species ranged from 8.8 mg/ℓ (98% of saturation), initially to 3.7 mg/ℓ (35% of saturation) at the end of exposure. The pH ranged from 7.0-7.4. Based on the 96-hour LC50's estimated for the fish species tested (Table 16), channel catfish appeared to be the most sensitive species to DIMP (lot #1), followed by bluegill, fathead minnow and rainbow trout. The 96-hour LC50's were 285, 406, 479 and 631 mg/ℓ, respectively. During the course of experimentation at Bionomics, it became necessary to utilize additional DIMP material, i.e., lot #2. Preliminary testing indicated a significant difference in toxicity existed between DIMP lot #1 and DIMP lot #2. The material tested as lot #2 was approximately 2X to 5X less toxic than lot #1. Conversations with the supplier of DIMP (Richmond Organics), and subsequent gas chromatographic analyses of the different lots by Richmond Organics indicated that all samples received were 98% pure, and that there was an impurity in all of the samples tested. This impurity was identified as diisopropylphosphite (DIPP), and this appeared to be present at a higher concentration in

DIMP lot #2. Subsequent screening of DIPP with fathead minnow to a nominal concentration of 100 mg/ℓ produced no adverse effects on these fish. At the highest concentration of DIMP (lot #2) tested, the theoretical concentration of DIPP would be 64 mg/ℓ, which appeared to indicate that the variance in toxicity of the two lots of DIMP was not due to DIPP. However, this does not preclude any synergistic or additive effects. Fortunately, all tests were completed with lot #1, and lot #2 was used only as a check on previous data. It is interesting to note that the lot #1 DIMP exhibited increasing toxicity over time, while lot #2 appeared to exhibit the majority of its toxicity during the first 24 hours of exposure (Table 17).

Dissolved oxygen values for the Task 2 egg and fry bioassays ranged from 8.9 mg/ℓ (99% of saturation) initially to 3.9 mg/ℓ (43% of saturation) at the end of the tests. Observations during the bioassays with eggs were made at 24-hour intervals through 144 hours of exposure and percent hatch at that time was also recorded (Tables 18 and 19). Lot #2 appeared to be less toxic than lot #1 DIMP which is consistent with that data generated for Task 1. However, while being less toxic, the lot #2 DIMP appeared to greatly reduce hatch at five days when compared to controls. The 1-hour old and 7-day old fry exhibited similar sensitivities to DIMP

(lot #1) and the 30-day and 60-day old fry exhibited slightly greater sensitivities. The 96-hour LC50 values were >1,000 mg/ℓ for both 1-hour and 7-day old fry, and 635 mg/ℓ and 641 mg/ℓ for the 30- and 60-day old fry, respectively. In the 30- and 60-day old fry bioassays, lot #1 DIMP exhibited increasing toxicity over time, as was evident in those fish bioassays (Task 1) utilizing lot #1 DIMP.

Dissolved oxygen values measured in bioassays evaluating the effect of different water quality parameters ranged from 8.8 mg/ℓ (98% of saturation) to 3.5 mg/ℓ (39% of saturation) at the end of the tests. The pH values for all parameters except the pH parameters (pH 6.0 and pH 8.0) ranged from 6.8 to 7.5. The initial and final pH values for the pH 6.0 test were 6.2 and 6.3. The pH values for the pH 8.0 test ranged from 7.4 to 8.0. The results of acute bioassays indicate the toxicity of DIMP to bluegill was similar over the range of 15-25^{o}C (Tables 20 and 21). The higher temperature (25^{o}C) appeared to increase the toxicity of DIMP through the 24- and 48-hour time periods, with a lesser effect at 96 hours of exposure. This may be attributable to an increased metabolism of the fish at the higher temperature, or a greater solubility of the DIMP at this temperature. There appeared to be a general lessening of toxicity as the hardness increased. The 96-hour LC50 of the 35 mg/ℓ hardness water was

438 mg/ℓ, while those for the 100 mg/ℓ and 250 mg/ℓ hard water were 849 mg/ℓ and >1,000 mg/ℓ, respectively. Increased pH (8.0) appeared to reduce the toxicity of DIMP to comparable levels (96-hour LC50, >750<1,000).

Dissolved oxygen levels monitored during Task 4 (Stability of Toxicological Properties) appeared to remain constant throughout the "aging" periods, and decreased normally after introduction of the test organisms from 8.8 mg/ℓ (98% of saturation) to 2.8 mg/ℓ (31% of saturation) indicating that DIMP did not generate any significant oxygen demand. The pH values ranged from 6.9 to 7.5. In general, the data indicate that solutions of DIMP "aged" for 96 hours are approximately half as toxic to bluegill as unaged solutions (Tables 22 and 23) after 96 hours of exposure, while those solutions "aged" for shorter periods (0, 8. 24 and 48 hours of exposure) appeared as toxic at 96 hours of exposure as observed for bluegill in the other bioassays.

Those bluegill in Task 5 (Bioconcentration) exposed to 150 mg/ℓ ^{14}C-DIMP appeared normal, fed readily, and generally showed no signs of stress due to chemical toxicity. The mean measured concentration of ^{14}C-DIMP in the water through 14 days of exposure was 166.7 $\pm$ 13.6 mg/ℓ (nominal concentration 150.0 mg/ℓ).

The results of the analyses of edible portions of bluegill sampled during the 14 days of exposure are summarized in Table 24. Radiometric analyses indicate that the mean measured concentration of ^{14}C-residues remains below minimum detectable limits (100 mg/kg) throughout the entire observation period, and clearly indicates that bioconcentration of DIMP by bluegill does not occur.

Dicyclopentadiene (DCPD)

A summary of those calculated EC50 and LC50 values and 95% confidence limits at termination of exposure is presented (Table 25). Based on *in vivo* chlorophyll *a* reduction, the 96-hour EC50's for *M. aeruginosa*, *A. flos-aquae*, and *N. pelliculosa* were 31, 60 and 51 mg/ℓ , respectively. The 96-hour EC50 for *S. capricornutum* was >1,000 mg/ℓ, the highest concentration at which DCPD could be maintained in solution (Table 26).

The 96-hour EC50 values based on reduction of cell numbers of *M. aeruginosa*, *A. flos-aquae*, and *N. pelliculosa* were 31, 22 and 53 mg/ℓ , respectively, with the value for *S. capricornutum* again >100 mg/ℓ (Table 27).

At most test concentrations, DCPD produced a decrease in both chlorophyll *a* and cell numbers of exposed *M. aeruginosa* and *N. pelliculosa*. Both cell numbers and chlorophyll *a* content for *A. flos-aquae* and *S. capricornutum* appeared to be stimu-

lated by exposure to lower concentrations (10, 16, 25 and 40 mg/ℓ) of DCPD and inhibited at higher concentrations ($\geq$ 56 mg/ℓ). The toxicity of DCPD to S. capricornutum appeared to decrease with time (Tables 28-35). Initial and final pH values for controls and test concentrations ranged from 8.0 to 8.4 for all tests.

Of the macroinvertebrate species tested, D. magna appeared to be the most sensitive species, followed by A. militaris, and G. fasciatus (Table 36). The 48-hour LC50 values were 10.5, 15.0 and 21.2 mg/ℓ, respectively. The calculated 48-hour LC50 for C. tentans was 120 mg/ℓ, which indicates DCPD is approximately 10X less toxic to midges than to the other invertebrates tested during acute exposure. The DCPD appeared to exhibit the majority of its toxicity to all species during the first 24 hours of exposure (Table 37).

The dissolved oxygen concentrations measured in bioassays with all fish species from selected concentrations ranged from 8.8 mg/ℓ (98% of saturation) to 3.8 mg/ℓ (42% of saturation). The pH values ranged from 6.9 to 7.3 for all tests. As with macroinvertebrate tests, the majority of the toxicity of DCPD was observed during the first 24 hours of exposure (Tables 38 and 39).

Dissolved oxygen values measured during bioassays with fathead

minnow for the egg and fry tests ranged from 8.9 mg/ℓ (99% of saturation) initially to 3.3 mg/ℓ (37% of saturation) at the end of the tests. Initial and final pH values ranged from 6.9 to 7.3. The fathead minnow eggs at 144 hours of exposure appeared to be unaffected at concentrations as high as 1,000 mg/ℓ, exhibiting an 144-hour LC50 value of 2,390 mg/ℓ (Table 40 and 41). By comparison, the 1-hour and the 7-day old fry exhibited a greatly increased sensitivity, with 96-hour LC50 values of >21.0<24.0 mg/ℓ and 12.0 mg/ℓ, while the 30- and 60-day old fry exhibited similar but decreased susceptibilities at 96-hours (86.3 and 103 mg/ℓ, respectively). The eggs exhibit sensitivities approximately 75X less than that observed for the fathead minnow during the completion of Task 1 (31.1 mg/ℓ at 96 hours). This decreased sensitivity may be due to the impermeability of the outer membrane of the eggs at this time.

The concentration of dissolved oxygen measured during those bioassays conducted under varying conditions of water quality ranged from 8.9 mg/ℓ (99% of saturation) initially to 3.4 mg/ℓ (38% of saturation) at the end of the tests. The pH values for all parameters except pH 6.0 and pH 8.0 ranged from 6.9 to 7.4. The values for the pH 6.0 test ranged from 6.1 to 6.4; while those for the pH 8.0 parameter ranged from 7.5 to 8.3.

The 96-hour LC50 values observed for bluegill exposed to DCPD under various conditions of water quality appeared to be similar to the LC50's generated for bluegill in Task 1, with the possible exceptions of the 15^{o}C and 250 mg/ℓ hardness parameters (Tables 42 and 43). Exposure to DCPD at 15^{o}C resulted in an LC50 which was approximately 2X less than the LC50 generated for bluegill in Task 1.

However, the LC50's resulting from exposure of bluegill to DCPD in water of various qualities were generally similar. The very hard (250 mg/ℓ) water appeared to increase the toxicity of DCPD, with a 96-hour LC50 of 14.2 mg/ℓ. As in all of the previous bioassays, the toxic action of DCPD appeared to occur principally during the first 24 hours of exposure.

Dissolved oxygen concentrations measured in selected concentrations at 0, 48 and 96 hours of exposure for the Task 4 "Stability of Toxicological Properties" ranged from 8.8 mg/ℓ (98% of saturation) initially to 2.7 mg/ℓ (30% of saturation) at the end of the tests. The pH ranged from 6.7 to 7.4. The 96-hour LC50 values for bluegill exposed to DCPD utilizing various aged solutions appeared very similar, indicating little or no toxicity increase or decrease due to aging of test solutions (Tables 44 and 45).

Those bluegill exposed to 1.00 mg/ℓ ^{14}C-DCPD during the bioconcentration study (Task 5) appeared normal, fed readily, and generally showed no signs of stress due to chemical toxicity. The mean measured concentration of ^{14}C-DCPD in the water through 14 days of exposure was 0.98 $\pm$ 0.25 mg/ℓ.

The results of the analyses of edible portions of bluegill sampled during the 14 days of exposure are summarized in Table 46. Radiometric analyses indicate that the mean measured concentration of ^{14}C-residue was 50.73 _ 6.43 mg/kg and was calculated for the period of apparent equilibrium (days 2-4). The estimated maximum bioconcentration factor for bluegill exposed to ^{14}C-DCPD is 53X. Between this period and the end of the exposure (day 14), the mean measured ^{14}C-residues appeared to generally decrease. Within 24 hours after transfer to flowing, ^{14}C-DCPD-free water, the mean measured concentration of ^{14}C-DCPD in the muscle portion of bluegill decreased to below the minimum detectable limit of 5.00 mg/kg.

In terms of compounds known to be hazardous to the aquatic environment (e.g., DDT, dieldrin, heptachlor, hexachlorobenzene, Aroclor 1254, dioxin, methyl mercury, toxaphene), all of which have demonstrated bioconcentration factors of >8,000X and time

to reach equilibrium of >14 days (Macek *et al.*, 1975), it appears that the potential of DCPD to bioconcentrate is slight.

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Table 1 -- Physical characteristics of two compounds evaluated in bioassays conducted at E G & G, Bionomics. The diisopropylmethyl phosphonate (DIMP) was received from Richmond Organics, Richmond, Virginia. The dicyclopentadiene (DCPD) was received from Aldrich Chemical Company, Milwaukee, Wisconsin.

Compound	Physical characteristics	Date received	Identifying Number	Percent Activities
DIMP[a]	clear liquid	8/21/75	A00 G	98.5%
DIMP[a]	clear liquid	10/10/75	-	98.5%
DIMP[b]	clear liquid	1/23/76	-	98.5%
DIMP[c]	clear liquid	2/2/76	Lot #L-78	98.5%
DIMP[c]	clear liquid	2/24/76	-	98.5%
DCPD	clear liquid	8/29/75	022457	95%

[a] Tested as lot #1.

[b] Tested as lot #2.

[c] Not tested.

TABLE 2. RECOMMENDED RECONSTITUTED FRESH WATERS

Table 2a -- Quantities of reagent-grade chemicals required to prepare recommended reconstituted fresh waters and the resulting water qualities.

Name	Salts required (mg/l)				Water quality of reconstituted fresh water		
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl	pH[a]	Hardness[b]	Alkalinity[c]
very soft	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
soft	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
hard	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
very hard	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

Table 2b -- Quantities of reagent-grade chemicals to be added to aerated soft reconstituted fresh water for buffering pH. The solutions were not aerated after addition of these chemicals.

pH[c]	Milliliters of solution for 15 liters of water		
	1.0 N NaOH	1.0 M KH_2PO_4	0.5 M H_3BO_3
6.0	1.3	80.0	-
6.5	5.0	30.0	-
7.0	19.0	30.0	-
7.5	-	-	-
8.0	19.0	20.0	-
8.5	6.5	-	40.0
9.0	8.8	-	30.0
9.5	11.0	-	20.0
10.0	16.0	-	18.0

a Approximate equilibrium pH after aeration and with fish in water.

b Expressed in mg/ℓ as $CaCO_3$.

c Approximate equilibrium pH with fish in water.

Table 3 -- Summary of calculated EC50 and LC50 values and 95% confidence limits for species of algae, invertebrates, and fish (of various life stages or under different water quality conditions) exposed to diisopropylmethyl phosphonate (DIMP, lot #1) at termination of exposure.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)	
	96-hour EC50	
Algae Species	Decrease of chlorophyll a[a]	Decrease of cell numbers[b]
Microcystis aeruginosa[c]	1,827(714-4,677)[d]	2,234(942-5,283)
Anabeana flos-aquae[c]	6,334(4,649-8,644)	6,107(4,696-7,944)
Selenastrum capricornutum[c]	2,301(1,363-3,881)	2,623(1,297-5,311)
Navicula pelliculosa[c]	2,045(853-4,923)	2,345(740-5,946)
Invertebrate Species	48-hour LC50	
Daphnia magna[e]	267(222-321)	
Gammarus fasciatus[e]	494(404-605)	
Asellus militaris[e]	2,160(1,760-2,640)	
Chironomus tentans[e]	1,720(1,260-2,330)	
Fish Species	96-hour LC50	
Lepomis macrochirus[f]	406(359-459)	
Ictalurus punctatus[f]	285(224-362)	
Pimephales promelas[f]	479(408-563)	
Salmo gairdneri[f]	631(538-740)	

Table 3 -- Continued.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)
Fish/Life Stages	144-hour LC50
Pimephales promelas/ eggs[g]	475(285-793)
	96-hour LC50
Pimephales promelas/ 1-hour fry[g]	>1,000
Pimephales promelas/ 7-day fry[g]	>1,000
Pimephales promelas/ 30-day fry[g]	635(524-769)
Pimephales promelas/ 60-day fry[g]	641(548-749)
Fish/Water Quality	96-hour LC50
Lepomis macrochirus/ 15°C	464(381-565)
Lepomis macrochirus/ 20°C	481(394-588)
Lepomis macrochirus/ 25°C	257(202-328)
Lepomis macrochirus/ 35 mg/ℓ hardness	438(361-532)
Lepomis macrochirus/ 100 mg/ℓ hardness	849(727-993)
Lepomis macrochirus/ 250 mg/ℓ hardness	>1,000
Lepomis macrochirus/ pH 6.0	527(441-631)

Table 3 -- Continued.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)
Lepomis macrochirus/ pH 7.0	435(353-537)
Lepomis macrochirus/ pH 8.0	>750<1,000
Fish/Age of Solution	96-hour LC50
Lepomis macrochirus/ 0-hour[h]	353(290-430)
Lepomis macrochirus/ 8-hour[h]	405(299-550)
Lepomis macrochirus/ 24-hour[h]	425(315-572)
Lepomis macrochirus/ 48-hour[h]	452(368-572)
Lepomis macrochirus/ 96-hour[h]	>750<1,000

[a] Criterion for effect was reduction of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

[b] Criterion for effect was decrease in number of cells of exposed algae as compared to controls.

[c] Bioassays conducted at 21 $\pm$ 1.0°C.

[d] 95% confidence interval.

[e] Bioassays conducted at 20 $\pm$ 1.0°C.

[f] Bioassays conducted at 21 $\pm$ 1.0°C for the bluegill, channel catfish, and fathead minnow, and at 12 $\pm$ 1.0°C for the rainbow trout.

[g] Bioassays conducted at 25 $\pm$ 1.0°C.

[h] Bioassays conducted at 21 $\pm$ 1.0°C.

Table 4 -- Calculated 96-hour EC50's for *Microcystis aeruginosa*, *Anabean flos-aquae*, *Selenastrum capricornutum* and *Navicula pelliculosa* exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effect was reduction of *in vivo* chlorophyll *a* of exposed algae as compared to controls after 96 hours of exposure.

Species	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
M. aeruginosa	1,827	714-4,677
A. flos-aquae	6,334	4,649-8,644
S. capricornutum	2,301	1,363-3,881
N. pelliculosa	2,045	853-4,923

Table 5 -- Calculated 96-hour EC50's for *Microcystis aeruginosa*, *Anabeana flos-aquae*, *Selenastrum capricornutum*, and *Navicula pelliculosa* exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effect was decrease in number of cells of exposed algae as compared to controls after 96 hours of exposure.

Species	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
M. aeruginosa	2,234	942-5,283
A. flos-aquae	6,107	4,696-7,944
S. capricornutum	2,623	1,297-5,311
N. pelliculosa	2,345	740-5,946

Table 6 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Microcystis aeruginosa* during, and cell numbers (% relative to controls) after, 96 hour exposures to diisopropylmethyl phosphonate (DIMP, lot #1) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	-[a]	-	-	-
303	-2	-7	-8	-3
947	-7	-9	-22	-21
1,704	-12	-31	-41	-38
3,030	-25	-45	-55	-51
5,302	-38	-55	-90	-92
9,468	-46	-73	-95	-91
17,028	-47	-82	-97	-95
30,298	-62	-88	-100	-100

[a] Represents unity; percentage calculated.

Table 7 -- Calculated EC50's for Microcystis aeruginosa exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	14,297	2,073-98,467
48	4,223	1,316-13,539
96	1,827	714-4,677

Table 8 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Anabeana flos-aquae* during, and cell numbers (% relative to controls) after, 96 hour exposures to diisopropylmethyl phosphonate (DIMP, lot #1) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	-[a]	-	-	-
3,787	0	+1	-4	-6
4,734	0	+2	-21	-14
5,965	-3	-29	-38	-30
7,480	-11	-63	-87	-91
9,468	-40	-83	-95	-98
11,930	-53	-87	-91	-95
15,149	-47	-89	-98	-95

[a] Represents unity; percentage calculated.

Table 9 -- Calculated EC50's for Anabeana flos-aquae exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	13,066	6,874-24,711
48	6,950	3,266-14,202
96	6,334	4,649-8,644

Table 10 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Selenastrum capricornutum* during, and cell numbers (% relative to controls) after, 96 hour exposures to diisopropylmethyl phosphonate (DIMP, lot #1) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	48-hour	96-hour
control	-[a]	-	-	-
303	-2	-2	-2	+4
947	-5	-4	-5	+1
1,278	-7	-12	-20	-12
1,704	-5	-28	-39	-29
3,030	-25	-65	-71	-73
5,302	-20	-60	-89	-80
7,101	-32	-71	-95	-88
9,468	-60	-91	-99	-96

[a] Represents unity; percentage calculated.

Table 11 -- Calculated EC50's for Selenastrum capricornutum exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effects was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	12,876	1,335-124,030
48	3,304	1,515-7,186
96	2,301	1,363-3,882

Table 12 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Navicula pelliculosa* during, and cell numbers (% relative to controls) after, 96 hour exposures to diisopropylmethyl phosphonate (DIMP, lot #1) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	-[a]	-	-	-
303	0	-4	-3	-6
947	0	-17	-31	-33
3,030	-2	-24	-50	-46
3,977	0	-47	-69	-60
5,302	-12	-59	-65	-62
7,101	-46	-87	-98	-97
9,468	-87	-98	-100	-100

[a] Represents unity; percentage calculated.

Table 13 -- Calculated EC50's for *Navicula pelliculosa* exposed to diisopropylmethyl phosphonate (DIMP, lot #1). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	7,006	447-10,983
48	2,973	1,212-6,448
96	2,045	853-4,923

Table 14 -- Acute toxicity[a] of diisopropylmethyl phosphonate (DIMP, lot #1) to water flea[b] (*Daphnia magna*), scud[c] (*Gammarus fasciatus*), sowbug[d] (*Asellus militaris*), and midge larvae[e] (*Chironomus tentans*). These data are based on results of bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Species	LC50 (mg active ingredient/ℓ) 24-hour	LC50 (mg active ingredient/ℓ) 48-hour	No discernible effect level at 48 hours (mg/ℓ)
D. magna	>420	267 (222-321)[f]	180
G. fasciatus	1,070 (584.0-1,950)	494.0 (404.0-605.0)	320
A. militaris	3,620 (1,820-7,160)	2,160 (1,760-2,640)	-[g]
C. tentans	3,700 (1,800-7,930)	1,720 (1,260-2,330)	-

[a] All bioassays conducted at 20 ± 1.0°C.

[b] Water flea ≤ 24 hours old at the initiation of testing.

[c] Scud in juvenile state at the initiation of testing.

[d] Sowbug in juvenile stage at the initiation of testing.

[e] Midge larvae in second-third instar at initiation of testing.

[f] 95% confidence interval.

[g] Mortality observed in all concentrations (1,200-3,700 ppm) tested.

Table 15 -- Concentrations tested and corresponding observed percentage mortalities (based on the average of 3 replicate exposures) for water flea (*Daphnia magna*), scud (*Gammarus fasciatus*), sowbug (*Asellus militaris*), and midge larvae (*Chironomus tentans*) exposed to diisopropylmethyl phosphonate (DIMP, lot #1) after 24 and 48 hours.

Species	Nominal concentration (mg active ingredient/ℓ)	% mortality observed	
		24-hour	48-hour
D. magna	420	0	100
	320	0	90
	240	0	20
	180	0	0
	140	0	0
	control	0	0
G. fasciatus	1,200	67	100
	1,000	40	100
	750	13	100
	560	7	33
	420	13	27
	320	0	0
	control	0	0

Table 15 -- Continued.

Species	Nominal concentration (mg active ingredient/ℓ)	% mortality observed 24-hour	% mortality observed 48-hour
A. militaris	3,700	47	100
	2,800	20	60
	2,100	7	27
	1,600	0	40
	1,200	0	20
	control	0	0
C. tentans	3,700	53	100
	2,800	7	87
	2,100	7	53
	1,600	0	40
	1,200	0	20
	control	0	0

Table 16 -- Acute toxicity of lot #1 and lot #2 of diisopropylmethyl phosphonate (DIMP) to bluegill[a] (Lepomis macrochirus), channel catfish[b] (Ictalurus punctatus), fathead minnow[c] (Pimephales promelas) and rainbow trout[d] (Salmo gairdneri) after 24, 48 and 96 hours of exposure. These data are based on bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachsuetts.

Lot #/species	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
lot #1/ bluegill	>1,000	500 (419-596)[e]	406 (359-459)	320
lot #2/ bluegill	1,780 (1,580-2,010)	1,700 (1,500-1,930)	1,570 (1,370-1,800)	1,400
lot #1/ channel catfish	>1,000	>650<750	285 (224-362)	240
lot #2/ channel catfish	>1,800<2,100	>1,800<2,100	<1,400	-[f]
lot #1/ fathead minnow	>1,000	>1,000	479 (408-563)	320
lot #2/ fathead minnow	1,770 (1,320-2,370)	1,770 (1,320-2,370)	1,770 (1,320-2,370)	1,400
lot #1/ rainbow trout	>1,000	>750<1,000	631 (538-740)	560

Table 16 -- Continued.

Lot #/species	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
lot #2/ rainbow trout	1,820 (1,360-2,420)	1,540 (1,210-1,960)	1,300 (875-1,950)	870

[a] Bioassay conducted at 21 ± 1.0°C, mean wet weight of bluegill was 1.1 g.

[b] Bioassays conducted at 21 ± 1.0°C, mean wet weight of channel catfish was 1.3 g.

[c] Bioassays conducted at 21 ± 1.0°C, mean wet weight of fathead minnow was 1.4 g.

[d] Bioassays conducted at 12 ± 1.0°C, mean wet weight of rainbow trout was 1.6 g.

[e] 95% confidence interval.

[f] Mortality observed in all concentrations (1,400-3,200) tested.

Table 17 -- Concentrations tested and corresponding observed percentage mortalities for bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), fathead minnow (Pimephales promelas) and rainbow trout (Salmo gairdneri) exposed to lot #1 and lot #2 diisopropylmethyl phosphonate (DIMP) for 24, 48 and 96 hours.

Lot #/species	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour
lot #1/ bluegill	1,000	0	100	100
	750	0	100	100
	560	0	50	100
	420	0	40	70
	370	0	0	20
	320	0	0	0
	control	0	0	0
lot #2/ bluegill	2,400	100	100	100
	1,800	30	70	100
	1,600	30	70	70
	1,400	0	0	0
	1,000	0	0	0
	control	0	0	0

Table 17 -- Continued.

Lot #/species	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour
lot #1/ channel catfish	1,000	0	100	100
	750	0	100	100
	650	0	0	100
	370	0	0	100
	280	0	0	70
	240	0	0	0
	control	0	0	0
lot #2/ channel catfish	3,200	100	100	100
	2,400	100	100	100
	2,100	100	100	100
	1,800	0	0	100
	1,400	0	0	100
	control	0	0	0
lot #1/ fathead minnow	1,000	0	0	100
	750	0	0	100
	560	0	0	30
	420	0	0	30
	370	0	0	20
	320	0	0	0
	control	0	0	0

Table 17 -- Continued.

Lot #/species	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour
lot #2/ fathead minnow	3,200	100	100	100
	2,400	100	100	100
	1,800	70	70	70
	1,400	0	0	0
	control	0	0	0
lot #1/ rainbow trout	1,000	0	70	100
	750	0	0	100
	650	0	0	90
	560	0	0	0
	420	0	0	0
	control	0	0	0
lot #2/ rainbow trout	3,200	100	100	100
	2,400	100	100	100
	1,800	30	30	100
	1,400	0	30	30
	870	0	0	0
	control	0	0	0

Table 18 -- Acute toxicity of diisopropylmethyl phosphonate (DIMP) lot #1 and lot #2 to fathead minnow[a] (Pimephales promelas) at selected life stages. These data are based on results of bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Lot #/ life stage	LC50 (milligrams active ingredient/liter)				No discernible effect level at 96 hours (mg/ℓ)
	24-hour	48-hour	96-hour	144-hour	
lot #1/ eggs	>1,000	>1,000		475 (285-793)[b]	100
lot #2/ eggs	>4,200<5,600	5,140 (3,950-7,410)		2,250 (1,630-3,100)	-[c]
lot #1/ 1-hour old	>1,000	>1,000	>1,000		1,000
lot #2/ 1-hour old	>1,800<2,100	>1,800<2,100	>1,800<2,100		1,800
lot #1/ 7-day fry	>1,000	>1,000	>1,000		560
lot #1/ 30-day fry	>750<1,000	753 (552-1,030)	635 (524-769)		420

Table 18 -- Continued.

Lot #/ life stage	LC50 (milligrams active ingredient/liter)				No discernible effect level at 96 hours (mg/ℓ)
	24-hour	48-hour	96-hour	144-hour	
lot #1/ 60-day fry	>1,000	>650<750	641 (548-749)		560

[a] Bioassay conducted at 25 ± 1.0°C, fathead minnow in varying life stages at initiation of exposure.

[b] 95% confidence interval.

[c] Mortality observed in all concentrations tested.

Table 19 -- Concentrations tested and corresponding observed percentage mortalities (and percentage hatch at 144 hours) for selected life stages of fathead minnow (Pimephales promelas) exposed to diisopropylmethyl phosphonate (DIMP) lot #1 and lot #2 for 24, 48 and 96 hours (eggs at 144 hours).

Lot #/ life stage	Nominal concentration (mg/ℓ)	% mortality observed				% hatch
		24-hour	48-hour	96-hour	144-hour	144-hour
lot #1/ eggs[a]	1,000	0	0		83	17
	750	0	0		50	23[b]
	560	0	0		63	33[b]
	420	0	0		37	47[b]
	320	0	0		73	27
	240	0	0		17	53[b]
	180	0	0		17	67[b]
	100	0	0		0	100
	control	0	0		0	100
lot #2/ eggs[a]	5,600	100	100		100	0
	4,200	0	73		100	0
	3,200	0	0		33	0[b]
	2,400	0	0		20	0[b]
	1,800	0	0		37	0[b]
	1,400	0	0		17	40[b]
	control	0	0		0	100

Table 19 -- Continued.

Lot #/ life stage	Nominal concentration (mg/ℓ)	% mortality observed				% hatch
		24-hour	48-hour	96-hour	144-hour	144-hour
lot #1/ 1-hour old	1,000	0	0	0		
	320	0	0	0		
	100	0	0	0		
	control	0	0	0		
lot #3/ 1-hour old[c]	3,200	100	100	100		
	2,800	100	100	100		
	2,400	100	100	100		
	2,100	100	100	100		
	1,800	0	0	0		
	1,400	0	0	0		
	control	0	0	0		
lot #1/ 7-day old fry[c]	1,000	10	10	15		
	750	0	15	25		
	560	0	0	0		
	420	0	0	0		
	320	0	0	0		
	control	0	0	0		

Table 19 -- Continued.

Lot #/ life stage	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour	144-hour	% hatch 144-hour
lot #1/ 30-day fry[c]	1,000	80	100	100		
	750	0	20	80		
	560	0	0	20		
	420	0	0	0		
	320	0	0	0		
	control	0	0	0		
lot #1/ 60-day fry[c]	1,000	20	100	100		
	750	0	100	100		
	650	0	0	60		
	560	0	0	0		
	420	0	0	0		
	control	0	0	0		

[a] Based on a mean of 3 replicate exposures.

[b] Remaining percentage of live animals were unhatched ("eyed-up").

[c] Based on a mean of 2 replicate exposures.

Table 20 -- Acute toxicity of diisopropylmethyl phosphonate (DIMP, lot #1) to bluegill[a] (*Lepomis macrochirus*) under varying conditions of water quality. These data are based on results of assays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Water quality	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
15°C	>1,000	>1,000	464 (381-565)[b]	320
20°C	>1,000	641 (528-779)	481 (394-588)	320
25°C	634 (521-773)	368 (298-433)	257 (202-328)	140
35 mg/ℓ hardness	>1,000	639 (518-789)	438 (361-532)	240
100 mg/ℓ hardness	>1,000	>1,000	849 (727-993)	750
250 mg/ℓ hardness	>1,000	>1,000	>1,000	1,000
pH 6.0	>1,000	686 (533-884)	527 (441-631)	320
pH 7.0	>1,000	571 (419-779)	435 (353-537)	240

Table 20 -- Continued.

Water quality	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
pH 8.0	>1,000	>750<1,000	>750<1,000	750

[a] Bioassays conducted under conditions of varying water quality, mean wet weight of bluegill was 1.0 g.

[b] 95% confidence interval.

Table 21 -- Concentrations tested and corresponding observed percentage mortalities for bluegill (Lepomis macrochirus) exposed to lot #1 diisopropylmethyl phosphonate (DIMP) for 24, 48 and 96 hours under varying water qualities.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
15°C	1,000	0	30	100
	750	0	0	100
	560	0	0	80
	420	0	0	50
	320	0	0	0
	control	0	0	0
20°C	1,000	0	100	100
	750	0	70	100
	560	0	20	50
	420	0	0	40
	320	0	0	0
	control	0	0	0
25°C	1,000	100	100	100
	750	70	100	100
	560	30	100	100
	420	0	80	100
	320	0	20	80
	240	0	0	10
	140	0	0	0
	control	0	0	0

Table 21 -- Continued.

Water quality	Nominal concentration (mg/ℓ)	% mortality obsered		
		24-hour	48-hour	96-hour
35 mg/ℓ hardness	1,000	0	100	100
	750	0	40	100
	560	0	50	70
	420	0	0	20
	320	0	0	10
	240	0	0	0
	control	0	0	0
100 mg/ℓ hardness	1,000	0	20	100
	870	0	0	80
	750	0	0	0
	560	0	0	0
	420	0	0	0
	control	0	0	0
250 mg/ℓ hardness	1,000	0	0	0
	750	0	0	0
	560	0	0	0
	420	0	0	0
	control	0	0	0

Table 21 -- Continued.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
pH 6.0	1,000	0	100	100
	750	0	10	100
	560	0	0	50
	420	0	20	40
	320	0	0	0
	control	0	0	0
pH 7.0	1,000	0	100	100
	750	0	100	100
	560	0	10	40
	420	0	0	40
	320	0	0	20
	240	0	0	0
	control	0	0	0
pH 8.0	1,000	0	50	60
	750	0	0	0
	560	0	0	0
	420	0	0	0
	control	0	0	0

Table 22 -- Concentrations tested and corresponding observed percentage mortalities for bluegill[a] (*Lepomis macrochirus*) exposed to diisopropylmethyl phosphonate (DIMP, lot #1) for 24, 48 and 96 hours utilizing various aged solutions. These data are based on bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Age of solution	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
0-hour	>1,000	>560<750	353 (290-430)[b]	240
8-hour	>1,000	547 (400-747)	405 (299-55)	320
24-hour	>1,000	541 (447-656)	425 (315-572)	320
48-hour	753 (552-1,030)	570 (454-715)	452 (368-572)	240
96-hour	>1,000	>1,000	>750<1,000	750

[a] Bioassays conducted at 21 ± 1.0°C, mean wet weight of bluegill was 1.1 g.

[b] 95% confidence interval.

Table 23 -- Acute toxicity of diisopropylmethyl phosphonate (DIMP, lot #1) to bluegill (*Lepomis macrochirus*) after 96-hours utilizing various aged solutions.

Age of solution	Nominal concentration (mg/ℓ)	percentage mortality 24-hour	48-hour	96-hour
0-hour	1,000	0	100	100
	750	0	100	100
	560	0	0	100
	420	0	0	70
	320	0	0	50
	240	0	0	0
	control	0	0	0
8-hour	1,000	20	100	100
	750	0	100	100
	560	0	60	100
	420	0	0	80
	320	0	0	0
	control	0	0	0
24-hour	1,000	50	100	100
	750	0	90	100
	560	0	50	100
	420	0	20	20
	320	0	0	0
	control	0	0	0

Table 23 -- Continued.

Age of solution	Nominal concentration (mg/ℓ)	percentage mortality		
		24-hour	48-hour	96-hour
48-hour	1,000	100	100	100
	750	20	90	100
	560	0	0	40
	420	0	10	20
	320	0	10	10
	240	0	0	0
	control	0	0	0
96-hour	1,000	10	30	80
	750	0	0	0
	560	0	0	0
	420	0	0	0
	320	0	0	0
	control	0	0	0

Table 24 -- Mean[a] concentrations of ^{14}C-diisopropylmethyl phosphonate[b] (DIMP, lot #2) measured in water and bluegill[c] (*Lepomis macrochirus*) during a 14-day exposure period and in bluegill during an additional 7-day depuration period following transfer of fish to flowing, ^{14}C-DIMP-free water.

	Day	Mean measured ^{14}C-residue concentration water (mg/ℓ)	bluegill (mg/kg)
Exposure	0	177.67	<100
	1	177.00	<100
	2	176.50	<100
	4	168.50	<100
	7	172.00	<100
	10	150.00	<100
	14	145.00	<100
		$\bar{x}$ = 166.67 (13.57)[d]	
Depuration	1		<100
	3		<100
	7		<100

[a] Mean based on two radiometric analyses for water and three radiometric analyses for fish.

[b] Minimum detectable limits = 7.5 mg/ℓ for water and 100 mg/kg for fish.

[c] Mean weight of bluegill was 3.0 g.

[d] Standard deviation (±).

Table 25 -- Summary of calculated EC50 or LC50 values and 95% confidence limits for species of algae, invertebrates, and fish (of various life stages or under different water quality conditions) exposed to dicyclopentadiene (DCPD) at termination of exposure.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)	
	96-hour EC50	
Algae Species	Decrease of chlorophyll *a*[a]	Decrease of cell numbers[b]
Microcystis aeruginosa[c]	31(17-57)[d]	31(20-48)
Anabeana flos-aquae[c]	60(37-90)	22(5-107)
Selenastrum capricornutum[c]	>100	>100
Navicula pelliculosa[c]	51(38-68)	53(40-70)
Invertebrate Species	48-hour LC50	
Daphnia magna[e]	10.5(8.40-13.2)	
Gammarus fasciatus[e]	21.2(17.4-26.0)	
Asellus militaris[e]	15.0(12.2-18.3)	
Chironomus tentans[e]	120(97.8-148)	
Fish Species	96-hour LC50	
Lepomis macrochirus[f]	23.3(17.6-30.1)	
Ictalurus punctatus[f]	15.7(13.8-18.0)	
Pimephales promelas[f]	31.1(23.0-42.0)	
Salmo gairdneri[f]	15.9(13.0-19.5)	

Table 25 -- Continued.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)
Fish/Life Stages	144-hour LC50
Pimephales promelas/ eggs[g]	2,390(1,920-2,960)
	96-hour LC50
Pimephales promelas/ 1-hour fry[g]	>21.0<24.0
Pimephales promelas/ 7-day fry[g]	12.0(10.0-14.2)
Pimephales promelas/ 30-day fry[g]	86.3(74.0-101)
Pimephales promelas/ 60-day fry[g]	103(62.5-171)
Fish/Water Quality	96-hour LC50
Lepomis macrochirus/ 15°C	47.5(37.5-60.2)
Lepomis macrochirus/ 20°C	31.7(23.5-42.8)
Lepomis macrochirus/ 25°C	37.6(33.7-42.0)
Lepomis macrochirus/ 35 mg/ℓ hardness	34.8(28.8-42.0)
Lepomis macrochirus/ 100 mg/ℓ hardness	>24.0<28.0
Lepomis macrochirus/ 250 mg/ℓ hardness	14.2(11.5-17.6)

Table 25 -- Continued.

Species	Calculated EC50 and LC50 values (mg active ingredient/ℓ)
Lepomis macrochirus/ pH 6.0	27.8(23.8-32.4)
Lepomis macrochirus/ pH 7.0	36.8(30.6-44.3)
Lepomis macrochirus/ pH 8.0	24.2(19.3-30.4)
Fish/Age of Solution	96-hour LC50
Lepomis macrochirus/ 0-hour[h]	20.0(25.7-33.1)
Lepomis macrochirus/ 8-hour[h]	23.4(17.2-31.9)
Lepomis macrochirus/ 24-hour[h]	27.1(22.6-32.4)
Lepomis macrochirus/ 48-hour[h]	34.6(28.2-42.4)
Lepomis macrochirus/ 96-hour[h]	44.3(36.0-54.6)

[a] Bioassays conducted at 21 ± 1.0°C.

[b] Criterion for effect was reduction *in vivo* chlorophyll *a* of exposed algae as compared to controls.

[c] 95% confidence interval.

[d] Criterion for effect was decrease in number of cells of exposed algae as compared to controls.

[e] Bioassays conducted at 20 ± 1.0°C.

[f] Bioassays conducted at 21 ± 1.0°C for the bluegill, channel catfish, and fathead minnow, and at 12 ± 1.0°C for the rainbow trout.

[g] Bioassays conducted at 25 ± 1.0°C.

[h] Bioassays conducted at 21 ± 1.0°C.

Table 26 -- Calculated 96-hour EC50's for *Microcystis aeruginosa*, *Anabeana flos-aquae*, *Selenastrum capricornutum*, and *Navicula pelliculosa* exposed to dicyclopentadiene (DCPD). Criterion for effect was reduction of *in vivo* chlorophyll *a* of exposed algae as compared to controls after 96 hours of exposure.

Species	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
M. aeruginosa	31	17-57
A. flos-aquae	60	37-90
S. capricornutum	>100	-[a]
N. pelliculosa	51	38-68

[a] Percentage effect insufficient to calculate the confidence limits.

Table 27 -- Calculated 96-hour EC50's for *Microcystis aeruginosa*, *Anabeana flos-aquae*, *Selenastrum capricornutum*, and *Navicula pelliculosa* exposed to dicyclopentadiene (DCPD). Criterion for effect was decrease in number of cells of exposed algae as compared to controls after 96 hours of exposure.

Species	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
M. aeruginosa	31	20-48
A. flos-aquae	22	5-107
S. capricornutum	>100	-[a]
N. pelliculosa	53	40-70

[a] Percentage effect insufficient to calculate confidence limits.

Table 28 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Microcystis aeruginosa* during, and cell numbers (% relative to controls) after, 96 hours exposures to dicyclopentadiene (DCPD) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	-[a]	-	-	-
10	-11	-17	-4	-1
16	-14	-26	-12	0
25	-32	-41	-40	-33
40	-51	-64	-66	-61
63	-79	-97	-88	-77
79	-68	-100	-100	-100
100	-84	-100	-100	-100

[a] Represents unity; percentage change calculated.

Table 29 -- Calculated EC50's for *Microcystis aeruginosa* exposed to dicyclopentadiene (DCPD). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	39	18-84
48	24	12-46
96	31	17-57

Table 30 -- In vivo chlorophyll a concentration (% relative to controls) in Anabeana flos-aquae during, and cell numbers (% relative to controls) after, 96-hour exposures to dicyclopentadiene (DCPD) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll a			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	-[a]	-	-	-
10	+54	+32	+47	+29
16	+59	+55	+58	+60
25	+42	+57	+61	+58
40	+12	+45	+51	+47
56	-4	-21	-38	-43
63	-29	-70	-70	-74
79	-57	-90	-87	-81
100	-68	-96	-97	-91

[a] Represents unity; percentage change.

Table 31 -- Calculated EC50's for *Anabeana flos-aquae* exposed to dicyclopentadiene (DCPD). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	82	57-116
48	61	44-86
96	60	37-90

Table 32 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Selenastrum capricornutum* during, and cell numbers (% relative to controls) after, 96 hour exposures to dicyclopentadiene (DCPD) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change: Chlorophyll *a* 24-hour	48-hour	96-hour	Cell no. 96-hour
control	-[a]	-	-	-
10	+23	+19	+16	+10
25	+18	+24	+29	+14
40	+21	+17	+38	+28
56	-33	-1	+21	+23
63	-27	-18	+11	+5
79	-49	-14	-7	-4
100	-66	-25	-4	-4

[a] Represents unity; percentage change.

Table 33 -- Calculated EC50's for Selenastrum capricornutum exposed to dicyclopentadiene (DCPD). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	80	40-166
48	>100	-[a]
96	>100	-

[a] Percentage effect insufficient to calculate confidence limits.

Table 34 -- *In vivo* chlorophyll *a* concentration (% relative to controls) in *Navicula pelliculosa* during, and cell numbers (% relative to controls) after, 96 hour exposures to dicyclopentadiene (DCPD) in a static bioassay.

Nominal concentration (mg/ℓ)	Percentage change			
	Chlorophyll *a*			Cell no.
	24-hour	48-hour	96-hour	96-hour
control	–[a]	–	–	–
10	-22	-7	0	+2
25	-27	-12	+4	+7
40	-31	-28	-18	-15
56	-67	-80	-70	-61
63	-60	-73	-76	-78
79	-69	-84	-91	-84
100	-75	-93	-100	-100

[a] Represents unity; percentage change.

Table 35 -- Calculated EC50 for Navicula pelliculosa exposed to dicyclopentadiene (DCPD). Criterion for effect was decrease of *in vivo* chlorophyll *a* of exposed algae as compared to controls.

Hour	96-hour EC50 (mg/ℓ)	95% confidence limits (mg/ℓ)
24	43	14-136
48	40	23-71
96	51	38-68

Table 36 -- Acute toxicity[a] of dicyclopentadiene (DCPD) to water flea[b] (*Daphnia magna*), scud[c] (*Gammarus fasciatus*), sowbug[d] (*Asellus militaris*), and midge larvae (*Chironomus tentans*). These data are based on results of bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Species	LC50 (mg active ingredient/ℓ) 24-hour	LC50 (mg active ingredient/ℓ) 48-hour	No discernible effect level at 48 hours (mg/ℓ)
D. magna	11.6 (9.21-14.2)[f]	10.5 (8.40-13.2)	5.30
G. fasciatus	30.2 (17.4-52.5)	21.2 (17.4-26.0)	11.4
A. militaris	15.6 (12.7-19.1)	15.0 (12.2-18.3)	9.50
C. tentans	153 (130-180)	120 (97.8-148)	56.0

[a] All bioassays conducted at 20 $\pm$ 1.0°C.

[b] Water flea $\leq$ 24 hours old at the initiation of testing.

[c] Scud in juvenile stage at the initiation of testing.

[d] Sowbug in juvenile stage at the initiation of testing.

[e] Midge larvae in second-third instar at initiation of testing.

[f] 95% confidence interval.

Table 37 -- Concentrations tested and corresponding observed percentage mortality (based on the average of 3 replicate concentrations) for water flea (*Daphnia magna*), scud (*Gammarus fasciatus*), sowbug (*Asellus militaris*), and midge larvae (*Chironomus tentans*) exposed to dicyclopentadiene (DCPD) after 24 and 48 hours.

Species	Nominal concentration (mg active ingredient/ℓ)	% mortality observed	
		24-hour	48-hour
D. magna	22.8	100	100
	17.1	80	87
	13.3	80	87
	9.50	60	67
	7.10	0	20
	5.30	0	0
	control	0	0
G. fasciatus	35.0	67	100
	26.6	20	40
	19.9	27	33
	15.2	7	7
	11.4	0	0
	control	0	0

Table 37 -- Continued.

Species	Nominal concentration (mg active ingredient/ℓ)	% mortality observed	
		24-hour	48-hour
A. militaris	30.4	100	100
	22.8	100	100
	17.1	20	40
	13.3	13	27
	9.50	0	0
	control	0	0
C. tentans	200	100	100
	180	40	73
	140	13	33
	100	0	20
	75.0	0	7
	56.0	0	0
	control	0	0

Table 38 -- Acute toxicity of dicyclopentadiene (DCPD) to bluegill[a] (Lepomis macrochirus), channel catfish[b] (Ictalurus punctatus), fathead minnow[c] (Pimephales promelas), and rainbow trout[d] (Salmo gairdneri) after 24, 48 and 96 hours of exposure. These data are based on bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Species	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
bluegill	25.9 (22.4-29.9)[e]	24.6 (21.7-27.9)	23.3 (17.6-30.1)	18.0
channel catfish	21.7 (18.4-25.5)	20.0 (17.2-23.3)	15.7 (13.8-18.0)	14.0
fathead minnow	32.0 (23.7-43.2)	31.1 (23.0-42.0)	31.1 (23.0-42.0)	24.0
rainbow trout	23.7 (19.3-29.2)	15.9 (13.0-19.5)	15.9 (13.0-19.5)	10.0

[a] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of bluegill was 1.1 g.

[b] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of channel catfish was 1.3 g.

[c] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of fathead minnow was 1.4 g.

[d] Bioassay conducted at 12 $\pm$ 1.0°C, mean wet weight of rainbow trout was 1.6 g.

[e] 95% confidence interval.

Table 39 -- Concentrations tested and corresponding observed percentage mortalities for bluegill[a] (*Lepomis macrochirus*), channel catfish[b] (*Ictalurus punctatus*), fathead minnow[c] (*Pimephales promelas*) and rainbow trout[d] (*Salmo gairdneri*) exposed to dicyclopentadiene (DCPD) for 24, 48 and 96 hours.

Species	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
bluegill	32.0	100	100	100
	28.0	20	90	100
	24.0	10	10	10
	18.0	0	0	0
	14.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
channel catfish	32.0	100	100	100
	24.0	60	60	100
	18.0	20	20	100
	16.0	0	40	60
	14.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

Table 39 -- Continued.

Species	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour
fathead minnow	56.0	100	100	100
	42.0	100	100	100
	32.0	20	60	60
	24.0	0	0	0
	18.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
rainbow trout	42.0	100	100	100
	32.0	60	100	100
	24.0	40	100	100
	18.0	40	40	40
	14.0	0	20	20
	10.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

[a] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of bluegill was 1.1 g.

[b] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of channel catfish was 1.3 g.

[c] Bioassay conducted at 21 $\pm$ 1.0°C, mean wet weight of fathead minnow was 1.4 g.

[d] Bioassay conducted at 12 $\pm$ 1.0°C, mean wet weight of rainbow trout was 1.6 g.

Table 40 -- Acute toxicity of dicyclopentadiene (DCPD) to fathead minnow[a] (Pimephales promelas) at selected life stages. These data are based on results of bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Life stage	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	144-hour	No discernible effect level at 144 hours (mg/ℓ)
eggs	>4,200<5,600	2,550 (2,040-3,180)[b]	-[c]	2,390 (1,920-2,960)	1,000
1-hour old fry	>21.0<24.0	>21.0<24.0	>21.0<24.0		21.0
7-day fry	12.7 (10.6-15.3)	12.7 (10.6-15.3)	12.0 (10.0-14.2)		7.5
30-day fry	86.3 (74.0-101)	86.3 (74.0-101)	86.3 (74.0-101)		75
60-day fry	103 (62.5-171)	103 (62.5-171)	103 (62.5-171)		75

[a] Bioassays conducted at 25°C, fathead minnow at various stages of development when exposure began.

[b] 95% confidence interval.

[c] LC50 not calculated.

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Table 41 -- Concentrations tested and corresponding observed percentage mortalities (and percentage hatch at 144 hours) for selected life stages of fathead minnow (Pimephales promelas) exposed to dicyclopentadiene (DCPD) for 24, 48 and 96 hours (eggs at 144 hours).

Life stage	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour	144-hour	% hatch 144-hour
eggs[a]	5,600	100	100		100	0
	4,200	0	77		83	17
	3,200	0	83		93	7
	2,400	0	10		23	77
	1,400	0	27		37	23[b]
	1,400	0	3		3	97
	1,000	0	0		0	100
	control (acetone)	0	0		0	100
	control	0	0		0	100
1-hour old[c]	37.0	100	100	100		
	28.0	100	100	100		
	24.0	100	100	100		
	21.0	0	0	0		
	16.0	0	0	0		
	control (acetone)	0	0	0		
	control	0	0	0		

Table 41 -- Continued.

Life stage	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour	144-hour	% hatch 144-hour
7-day fry[c]	18.0	100	100	100		
	14.0	20	20	20		
	10.0	10	10	10		
	8.7	0	0	20		
	7.5	0	0	0		
	control (acetone)	0	0	0		
	control	0	0	0		
30-day fry[c]	100	100	100	100		
	87.0	40	40	40		
	75.0	0	0	0		
	56.0	0	0	0		
	42.0	0	0	0		
	control (acetone)	0	0	0		
	control	0	0	0		
60-day fry[c]	180	100	100	100		
	100	80	80	80		
	75.0	0	0	0		
	56.0	0	0	0		
	42.0	0	0	0		
	control (acetone)	0	0	0		
	control	0	0	0		

[a] Based on a mean of 3 replicates.

[b] Remaining percent of live animals were unhatched ("eyed up").

[c] Based on a mean of duplicates.

Table 42 -- Acute toxicity of dicyclopentadiene (DCPD) to bluegill[a] (*Lepomis macrochirus*) under varying conditions of water quality. These data are based on results of bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Water quality	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
15°C	76.3 (55.9-104)[b]	60.4 (48.6-75.0)	47.5 (37.5-60.2)	32.0
20°C	35.5 (29.5-42.6)	35.5 (29.5-42.6)	31.7 (23.5-42.8)	24.0
25°C	42.6 (38.8-46.8)	37.6 (33.7-42.0)	37.6 (33.7-42.0)	28.0
35 mg/ℓ hardness	36.4 (30.4-37.9)	35.1 (29.1-42.3)	34.8 (28.8-42.0)	18.0
100 mg/ℓ hardness	33.0 (28.7-37.9)	>24.0<28.0	>24.0<28.0	24.0
250 mg/ℓ hardness	>32.0<42.0	17.8 (13.3-23.8)	14.2 (11.5-17.6)	7.5
pH 6.0	62.8 (51.7-76.3)	30.7 (23.1-40.9)	27.8 (23.8-32.4)	24.0
pH 7.0	47.8 (39.9-57.3)	45.4 (37.2-55.3)	36.8 (30.6-44.3)	24.0
pH 8.0	31.5 (23.5-42.5)	31.1 (23.0-42.0)	24.2 (19.3-30.4)	16.0

[a] Bioassay conducted under varying water quality, mean wet weight of bluegill was 1.0 g.

[b] 95% confidence interval.

Table 43 -- Concentrations tested and corresponding observed percentage mortalities for bluegill (Lepomis macrochirus) exposed to dicyclopentadiene (DCPD) for 24, 48 and 96 hours in varying water qualities.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
15°C	100	100	100	100
	75.0	10	20	50
	56.0	0	40	100
	42.0	0	10	40
	32.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
20°C	75.0	100	100	100
	56.0	100	100	100
	42.0	90	90	100
	32.0	20	20	30
	24.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

Table 43 -- Continued.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
$25^{o}C$	56.0	100	100	100
	49.0	80	100	100
	42.0	30	30	30
	37.0	10	20	20
	32.0	0	40	40
	28.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
35 mg/ℓ hardness	56.0	100	100	100
	42.0	80	90	90
	32.0	10	30	30
	24.0	0	0	40
	18.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
100 mg/ℓ hardness	42.0	100	100	100
	32.0	0	100	100
	28.0	30	100	100
	24.0	0	0	0
	18.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

Table 43 -- Continued.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed		
		24-hour	48-hour	96-hour
250 mg/ℓ hardness	42.0	100	100	100
	32.0	0	100	100
	24.0	0	100	100
	18.0	0	60	60
	14.0	0	0	10
	10.0	0	0	20
	7.5	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
pH 6.0	100	100	100	100
	75.0	80	100	100
	56.0	30	100	100
	42.0	0	90	100
	32.0	0	100	100
	28.0	0	0	30
	24.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

Table 43 -- Continued.

Water quality	Nominal concentration (mg/ℓ)	% mortality observed 24-hour	48-hour	96-hour
pH 7.0	75.0	100	100	100
	56.0	90	90	100
	42.0	10	60	60
	32.0	0	0	10
	24.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0
pH 8.0	42.0	100	100	100
	32.0	40	60	70
	24.0	0	0	10
	16.0	0	0	0
	12.0	0	0	0
	control (acetone)	0	0	0
	control	0	0	0

Table 44 -- Concentrations tested and corresponding observed percentage mortalities for bluegill[a] (Lepomis macrochirus) exposed to dicyclopentadiene (DCPD) for 24, 48 and 96 hours utilizing various aged solutions. These data are based on bioassays conducted at the Aquatic Toxicology Laboratory of E G & G, Bionomics, Wareham, Massachusetts.

Age of solution	LC50 (milligrams active ingredient/liter) 24-hour	48-hour	96-hour	No discernible effect level at 96 hours (mg/ℓ)
0-hour	41.8 (31.0-56.4)[b]	28.7 (23.6-35.0)	20.0 (25.7-33.1)	-[c]
8-hour	35.6 (29.3-43.2)	28.7 (23.7-35.4)	23.4 (17.2-31.9)	18.0
24-hour	30.8 (22.8-41.7)	27.1 (22.6-32.4)	27.1 (22.6-32.4)	18.0
48-hour	34.9 (28.6-42.7)	34.6 (28.2-42.4)	34.6 (28.2-42.4)	24.0
96-hour	47.5 (37.8-59.5)	44.3 (36.0-54.6)	44.3 (36.0-54.6)	24.0

[a] Bioassay conducted at 21 ± 1.0°C, mean wet weight of bluegill was 1.1 g.

[b] 95% confidence interval.

[c] Mortality at all concentrations tested.

Table 45 -- Acute toxicity of dicyclopentadiene (DCPD) to bluegill (*Lepomis macrochirus*) after 96 hours utilizing various aged solutions.

Age of solution	Nominal concentration (mg/ℓ)	Percentage mortality 0-hour	48-hour	96-hour
0-hour	56.0	100	100	100
	42.0	40	100	100
	32.0	0	30	70
	24.0	0	10	10
	18.0	0	0	10
	control	0	0	0
8-hour	56.0	100	100	100
	42.0	70	90	100
	32.0	40	70	100
	24.0	0	60	60
	18.0	0	0	0
	control	0	0	0
24-hour	56.0	100	100	100
	42.0	100	100	100
	32.0	70	90	90
	24.0	0	10	10
	18.0	0	0	0
	control	0	0	0

Table 45 -- Continued.

Age of solution	Nominal concentration (mg/ℓ)	Percentage mortality 0-hour	24-hour	96-hour
48-hour	56.0	100	100	100
	42.0	70	70	70
	32.0	60	70	70
	24.0	0	0	0
	18.0	0	0	0
	control	0	0	0
96-hour	75.0	100	100	100
	56.0	10	30	30
	42.0	20	50	50
	32.0	10	10	10
	24.0	0	0	0
	control	0	0	0

Table 46 -- Mean[a] concentration of ^{14}C-dicyclopentadiene (DCPD)[b] measured in water and bluegill[c] (Lepomis macrochirus) during a 14-day exposure period and in bluegill during an additional 7-day depuration period following transfer to flowing, ^{14}C-DCPD-free water.

	Day	Mean measured ^{14}C-residue concentration water (mg/ℓ)	bluegill (mg/kg)
Exposure	0	0.77	
	1	1.44	36.37(3.62)
	2	0.70	55.27(17.03)
	4	0.91	46.18(12.51)
	7	0.87	33.00(10.00)
	10	1.08	18.93(1.76)
	14	1.11	11.37(4.26)
		$\bar{x}$ 0.98 (0.25)[d]	
Depuration	1		<5.00
	3		<5.00
	7		<5.00

[a] Mean based on two radiometric analyses for water and three radiometric analyses for fish.

[b] Minimum detectable limits = 0.60 mg/ℓ for water and 5.00 mg/kg for fish.

[c] Mean wet weight of bluegill = 3.0 g.

[d] Standard deviation (±).

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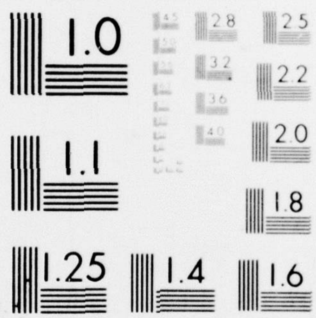

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